Arsenate Reductases in Prokaryotes and Eukaryotes

Rita Mukhopadhyay and Barry P. Rosen

Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, Michigan, USA

The ubiquity of arsenic in the environment has led to the evolution of enzymes for arsenic detoxification. An initial step in arsenic metabolism is the enzymatic reduction of arsenate [As(V)] to arsenite [As(III)]. At least three families of arsenate reductase enzymes have arisen, apparently by convergent evolution. The properties of two of these are described here. The first is the prokaryotic ArsC arsenate reductase of *Escherichia coli*. The second, Acr2p of *Saccharomyces cerevisiae*, is the only identified eukaryotic arsenate reductase. Although unrelated to each other, both enzymes receive their reducing equivalents from glutaredoxin and reduced glutathione. The structure of the bacterial ArsC has been solved at 1.65 Å. As predicted from its biochemical properties, ArsC structures with covalent enzyme–arsenic intermediates that include either As(V) or As(III) were observed. The yeast Acr2p has an active site motif $HC(X)_5R$ that is conserved in protein phosphotyrosine phosphatases and rhodanases, suggesting that these three groups of enzymes may have evolved from an ancestral oxyanion-binding protein. *Key words:* Acr2p, ArsC, arsenate reductase, arsenate resistance, glutaredoxin, phosphatase. *Environ Health Perspect* 110(suppl 5):745–748 (2002).
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Arsenic is classified as a carcinogen by the U.S. Environmental Protection Agency (U.S. EPA) (Smith et al. 1992). Exposure to arsenic in drinking water is associated with increased risk of skin, kidney, lung, and bladder cancers. Chronic effects of arsenic in the water supply include skin hyperpigmentation and keratoses of the hands and feet that frequently progress to skin cancers. In about 10% of these cases, exposure is associated with a very high incidence of lung, bladder, and other cancers. According to the National Resources Defense Council (NRDC), millions of Americans are consuming tap water every day that poses unacceptable cancer risks (NRDC 2001). Over 56 million Americans in the 25 reporting states consumed water from systems containing arsenic at levels presenting a potentially fatal cancer risk. In 1976 the U.S. EPA set the maximum contamination level (MCL) for arsenic at 50 μg/L. The National Research Council, the operating arm of the U.S. National Academy of Sciences, has recommended an MCL of 10 μg/L (U.S. EPA 2001), which will be put in place in the near future.

Arsenic enters the water supply primarily from geochemical sources (USGS 1998a). In Michigan, arsenic leaches into water from the Marshall Sandstone and Coldwater Shale (*Detroit Free Press* 1999; USGS 1998b). In regions of Oakland County the concentration of arsenic in water supplies greatly exceeds the current U.S. EPA MCL of 50 μg/L (*Detroit Free Press* 1999). Based on geology and limited sampling, 20–30% of water supply wells in Michigan have concentrations exceeding 10 μg/L.

Although in general arsenic enters the biosphere by leaching from geologic formations, anthropomorphic sources include arsenical-containing fungicides, pesticides, and herbicides. For example, in Novi, Michigan, 95 acres of land proposed for development are contaminated with arsenic from insecticides dating from when the land was used for orchards. In Michigan, chronic effects of arsenic include neurological effects similar to those of multiple sclerosis (*Detroit Free Press* 1997). It is likely that over a period of decades some of these effects will progress to various cancers (*Detroit Free Press* 1999). Thus, study of the mechanisms of arsenic detoxification in humans and other organisms is of considerable importance.

Convergent Evolution of Arsenate Reductases

Pathways for arsenic detoxification exist in all organisms examined, including bacteria and yeast (Bhattacharjee et al. 1999) (Figure 1). An initial step is biotransformation of arsenate [As(V)] to arsenite [As(III)] (Figure 1). Although As(V) can be reduced to As(III) nonenzymatically, this process is too slow to be physiologically significant, and organisms use enzymes that catalyze As(V) reduction (Bhattacharjee et al. 1999). These thiol-linked reductases are required to confer resistance to As(V) in both prokaryotes (Gladysheva et al. 1994; Ji et al. 1994) and eukaryotes
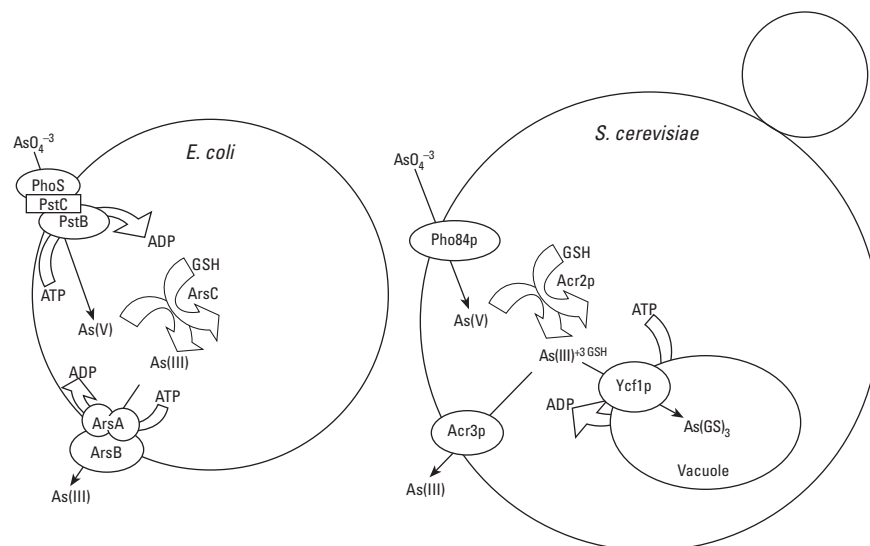
Figure 1. Pathways of arsenic detoxification in prokaryotes and eukaryotes. *E. coli*, a typical prokaryote, accumulates As(V) by phosphate transport systems such as the Pst ABC ATPase. As(V) is reduced to As(III) by ArsC, a Grx–GSH-linked enzyme. The resulting As(III) is pumped out of the cells by the ArsA/ArsB ATPase. The eukaryote *S. cerevisiae* uses a similar cycle of As(V) uptake, reduction, and extrusion from the cytosol, but the proteins that catalyze these reactions are the products of independent evolution from the bacteria. Phosphate transporters such as Pho84p accumulate As(V). Acr2p, a Grx–GSH-linked enzyme, reduces As(V) to As(III). As(III) can be extruded from the cells by the Acr3p carrier. Alternatively, As(III) can be conjugated with GSH to form $As(GS)_3$. The Ycf1p ABC ATPase removes As(III) from the cytosol by pumping $As(GS)_3$ into the vacuole.
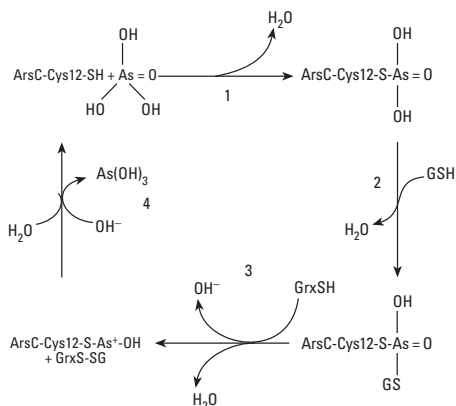
Figure 3. The reaction mechanism of the R773 ArsC. In the first step, an oxyanion [As(V), phosphate, or sulfate] is bound noncovalently to the enzyme. If the oxyanion is As(V), a covalent thiarsahydroxy intermediate forms with the thiolate of Cys12, as has been seen in the crystal structure of the As(V) complex. In the third step, a glutathionylated intermediate is formed that is then reduced to novel ArsC−S−As⁺−O intermediate observed in the enzyme–As(III) crystal structure. In the final step, this enzyme–As(III) complex dissociates to release free As(III).

(Bobrowicz et al. 1997; Mukhopadhyay and Rosen 1998). These enzymes arose independently at least three times by convergent evolution (Figure 2). One family includes the *Staphylococcus aureus* plasmid pI258–encoded *arsC* gene product (Ji et al. 1994). This ArsC enzyme is homologous to low molecular weight protein phosphotyrosine phosphatases (lmwPTPases) (Bennett et al. 2001). A second family includes the *Escherichia coli* plasmid R773 ArsC arsenate reductase (Rosen 1999). The third family includes the only known eukaryotic arsenate reductase, Acr2p from *Saccharomyces cerevisiae* (Bobrowicz et al.
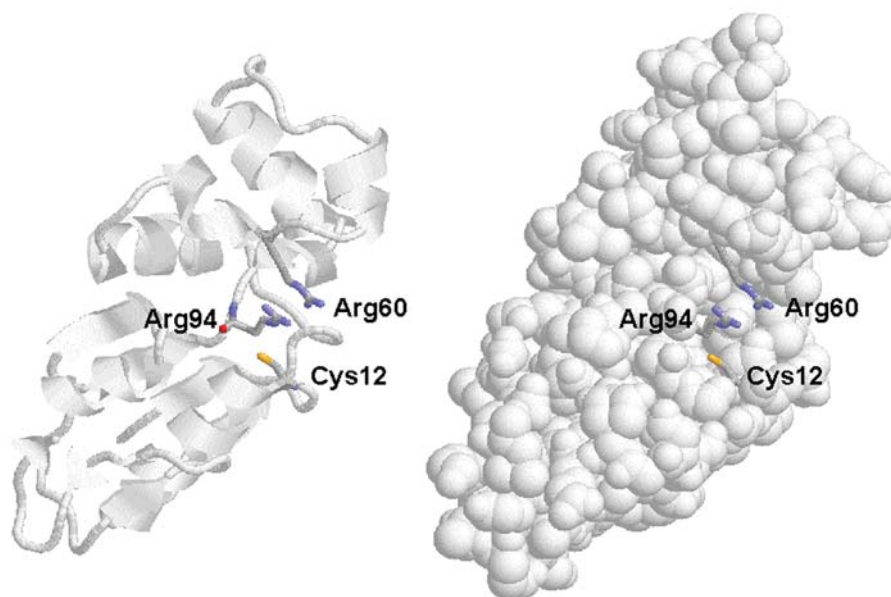
Figure 4. Crystal structure of the R773 ArsC at 1.65 Å. Two views are shown identifying secondary structural elements (left) or space filling (right). The active site includes the catalytic residue Cys12 and arginine residues Arg60 and Arg94, which are shown in stick form. The catalytic residue Cys12 is held in a rigid loop formed by two type I beta turns that bracket the cysteine (left). Arg94 forms a portion of the anion-binding site, and Arg60 stabilizes the covalent thiarsahydroxy intermediate.
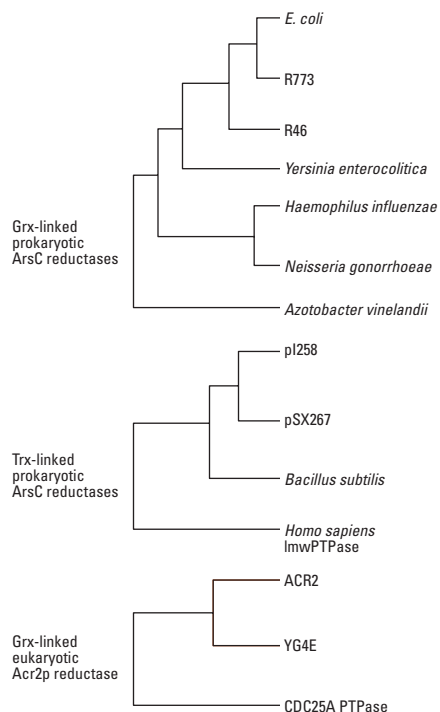

Figure 2. Three families of arsenate reductases. Reductases involved in As(V) detoxification have apparently evolved independently at least three times. One family is typified by the *E. coli* plasmid R773 ArsC that uses Grx and GSH as reductants. A second family represented by the *S. aureus* plasmid pI258 ArsC uses thioredoxin (Trx) as a reductant and is related to a family of lmwPTPases. Acr2p is the only eukaryotic arsenate reductase so far identified. It belongs to the superfamily of PTPases that includes the Cdc25a cell-cycle phosphatase.

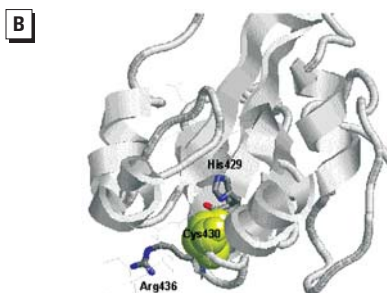Figure 5. Active site of the yeast Acr2p arsenate reductase. (*A*) Alignment of Acr2p with Yg4ep, an *S. cerevisiae* homolog, and the human Cdc25a. The consensus active site HC(X)₅R motif is identified. (*B*) Structure of the human Cdc25a. The active site Cys430 is identified, as is His429 that charge-pairs with Cys430, and Arg436, which forms part of the anion-binding loop. Acr2p is predicted to have a similar active site.

1997; Mukhopadhyay and Rosen 1998). Acr2p is homologous to the Cdc25a cell-cycle protein tyrosine phosphatase (PTPase) (Fauman et al. 1998) and to rhodanase, a thiosulfate sulfurtransferase (Hofmann et al. 1998). It is interesting that the substrates of all three types of enzymes, arsenate reductases, phosphatases and sulfurtransferases, are anions. This evolutionary relatedness suggests that the common ancestor had an oxyanion-binding site. We have studied ArsC from *E. coli* resistance plasmid R773 and the yeast Acr2p, and the properties of those two enzymes are described here in more detail.

ArsC: A Prokaryotic Arsenate Reductase

The R773 ArsC purified from *E. coli* requires reduced glutathione (GSH) and the small thiol transfer protein glutaredoxin (Grx) for arsenate reductase activity (Gladysheva et al. 1994). ArsC has a single catalytic cysteine residue, Cys12 (Liu et al. 1995), that forms a covalent thiolate–As(V) intermediate (Martin et al. 2001). Grx is required to reduce the enzyme-bound ES–As(V) intermediate to an ES–As(III) intermediate (Gladysheva et al. 1994; Shi et al. 1999). Members of the Grx family have two ways in which they can participate in thiol reduction reactions. First, they can catalyze reduction of intramolecular disulfide bonds (Bushweller et al. 1992). Because ArsC has only a single required cysteine residue, this seemed an unlikely mode of action of Grx. Second, Grx can participate in reduction of mixed disulfides between protein thiols and glutathione. The two modes can be differentiated by the use of appropriate Grx mutants. There are three glutaredoxins in *E. coli*, Grx1, Grx2, and Grx3 (Åslund et al. 1994), each of which can function in ArsC catalysis (Shi et al. 1999). Each has the consensus sequence Cys–Pro–Tyr–Cys. Mutants lacking the N-terminal cysteine are unable to participate in either mode of reduction. In contrast the C-terminal cysteine mutants function in reduction of mixed protein-glutathione disulfides but not reduction of intramolecular protein disulfide bonds (Bushweller et al. 1992). N-terminal cysteine mutants of Grx could not participate in ArsC-catalyzed As(V) reduction (Shi et al. 1999). On the other hand, Grx mutants lacking the C-terminal cysteine still supported ArsC activity. These results demonstrate that ArsC does not form an intramolecular disulfide during the catalytic cycle and are consistent with the existence of the mixed disulfide intermediate ArsC–S–SG. However, as described below, crystallographic data suggest that the intermediate could be a novel thiarsahydroxy ArsC–S–As(OH)–SG complex. An ArsC reaction scheme with the As(V) and As(III) intermediates is shown in Figure 3.

We have recently reported the X-ray crystal structures of ArsC at 1.65 Å (Martin et al. 2001). The active site can be seen to consist of the catalytic residue Cys12 and several arginine residues, including Arg60 and Arg94 (Figure 4). In addition to the native structure, structures of ArsC in complex with As(V) or As(III) were obtained. One structure is that of the covalent Cys12–S–As(V) adduct, which has a tetrahedral geometry with a sulfur–arsenic distance of 2.18 Å. The other structure is that of the Cys–S–As⁺–OH adduct.

Acr2p: A Eukaryotic Arsenate Reductase

Although it is likely that enzymes that catalyze As(V) reduction are ubiquitous, the only eukaryotic arsenate reductase identified is the 130-residue Acr2p protein from *S. cerevisiae* (Bobrowicz et al. 1997; Mukhopadhyay and Rosen 1998; Mukhopadhyay et al. 2000). The *ACR2* gene is required for As(V) resistance (Bobrowicz et al. 1997; Mukhopadhyay and Rosen 1998). Acr2p is apparently unrelated to bacterial arsenate reductases. On the other hand, it is a member of the superfamily of PTPases such as the human cell-cycle dual-specific phosphatase Cdc25a (Figure 5A). Members of this family have a consensus-active site $HC(X)_5R$ motif (Denu and Dixon 1995; Fauman et al. 1998) (Figure 5B). The consensus $C_{76}(X)_5R_{82}$ motif of Acr2p is likely to be part of the active site as well: either Cys76 or Arg82 mutations result in loss of As(V) resistance *in vivo* and As(V) reduction *in vitro* (Mukhopadhyay and Rosen 2001). These results suggest that Cys76 is the equivalent of Cys12 in ArsC and may form As(V) and As(III) intermediates. In support of this hypothesis, *in vitro* Acr2p exhibits the same Grx and GSH requirement as ArsC. All Grx proteins examined supported Acr2p activity, including a *S. cerevisiae* Grx and all three *E. coli* Grx proteins. Just as in the case of ArsC, the Grx C-terminal cysteine mutants functioned in As(V) reduction, indicating that Acr2p could have a catalytic cycle with an Acr2p–S–As(III)–SG intermediate (Mukhopadhyay et al. 2000).

Where are the mammalian arsenate reductases? Reductase activity has recently been reported in human liver (Radabaugh and Aposhian 2000), but it is not known whether the enzyme that catalyzes this activity is related to Acr2p or either of the bacterial enzymes. The *S. aureus* plasmid pI258 ArsC and the yeast Acr2p enzymes are both homologues of protein phosphotyrosine phosphatases (although to different families of phosphatase) (Bennett et al. 2001; Fauman et al. 1998). Acr2p is also homologous to rhodanases, which are thiosulfate sulfurtransferases (Hofmann et al. 1998). We suggest that all

arsenate reductases, whether eukaryotic or prokaryotic, share a common evolutionary lineage with phosphatases. Analysis of the phosphatase-like proteins identified in eukaryotic genomes may reveal the identity of mammalian arsenate reductase.

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